

## **Thioredoxin Reductase (TrxR) Activity Assay Kit**

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/Microplate Reader

**Cat No:** BC1155

**Size:**100T/96S

### **Components:**

**Reagent I:** 120 mL×1. Storage at 4°C.

**Reagent II:** 3mL×1. Storage at 4°C and protect from light. Dissolve with 5 mL of distilled water when the solution will be used.

**Reagent III:** Powder×2. Storage at 4°C. protect from light. Dissolve with 1.667 mL of distilled water when the solution will be used.

**Reagent IV:** 30μL×1. Storage at -20°C. Dilute Reagent IV with ethanol 10 times according to the number of samples before use.

### **Product Description:**

TrxR is a NADPH-dependent dimer selenase and includes FAD structure domain. TrxR belongs to pyridine nucleotide-disulfide REDOX enzyme, and form thioredoxin system with thioredoxin and NADPH. The activity of TrxR is similar with GR. TrxR could catalyzes GSSG reduce to GSH, which is the key enzyme in glutathione REDOX cycle.

TrxR catalyzes NADPH to reduceDTNB form TNB and NADP<sup>+</sup>, TNB has a absorbance at 412 nm, but reduced glutathione reacts with DTNB to form TNB, so the 2-Vinylpyridine in this kit can inhibit reduced glutathione in sample, the activity of TrxR can be calculated by detecting increase rate of TNB at 412 nm.

### **Reagents and Equipment Required but Not Provided:**

Spectrophotometer/microplate reader, ultra-micro glass cuvette/96 well flat-bottom plate,water bath, low temperature centrifuge, mortar/homogenizer, adjustable pipette,distilled water.

### **Procedure**

#### **I. Sample preparation:**

##### 1. Tissue:

Add 1 mL of Reagent I into 0.1 g of tissue, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4°C and take the supernatant on ice for test. Before test, mix supernatant and Reagent IV at a ratio of 50:1(add 2 μL of Reagent IV to 100 μL of supernatant), water bath at 37°C for 30 minutes, then keep on ice for test.

##### 2. Bacteria/cell:

Suggested 5 million with 1 mL of Reagent I, Splitting bacteria and cell with ultrasonic(ice bath,

power 300W, work time 3 s, interval 7 s, for 3 minutes), centrifuge at 10000 rpm and 4°C for 10 minutes. The supernatant on ice is used for test. before test, mix supernatant and Reagent IV at a ratio of 50:1 (add 2 μL of Reagent IV to 100 μL of supernatant), water bath at 37°C for 30 minutes, then keep on ice for test.

### III. Procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 412 nm, set the zero with distilled water.
2. Preheat reagent I at 37°C (mammal), 25°C (other) in water bath for 30 minutes.
3. Blank tube: take a micro glass cuvette/96 well flat-bottom plate, add 20 μL of Reagent II, 20 μL of Reagent III, 160 μL of Reagent I, mix them quickly, and then measure the absorbance at 412 nm for 10s. Take out the absorbance at 412 nm in a 37°C water bath for 5 min and record it as A1 and A2. Calculate  $\Delta A_B = A2 - A1$ .
4. Measuring tube: take a micro glass cuvette/96 well flat-bottom plate, add 20 μL of Reagent II, 20 μL of Reagent III, 140 μL of Reagent I, 20 μL of supernatant, mix it quickly, and then measure the absorbance at 412 nm for 10s, take out the absorbance at 412 nm quickly in a 37°C water bath for 5 min, and record it as A3 and A4.  $\Delta A_T = A4 - A3$ .

### III. Calculation:

#### A. micro glass cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every mg of protein.

$$\text{TrxR (U/mg prot)} = \frac{[\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv}}{(V_s \times C_{pr}) \div T} = 147 \times \frac{[\Delta A(T) - \Delta A(B)] \div C_{pr}}{T}$$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every gram of sample.

$$\text{TrxR (U/g)} = \frac{[\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv}}{(V_s \div V_{sv} \times W) \div T} = 147 \times \frac{[\Delta A(T) - \Delta A(B)] \div W}{T}$$

3. Cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every 10<sup>4</sup> cell.

$$\text{TrxR (U/10}^4\text{cell)} = \frac{[\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv}}{(N \div V_{sv} \times V_s) \div T} = 147 \times \frac{[\Delta A(T) - \Delta A(B)] \div N}{T}$$

$\epsilon$ : TNB molar extinction coefficient,  $1.36 \times 10^4$  L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{rv}$ : Total reaction volume, 200 μL =  $2 \times 10^{-4}$  L;

$V_s$ : Supernatant volume (mL), 0.02 mL;

$C_{pr}$ : Sample protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time (min), 5 minutes;

W: Sample weight (g);

V<sub>sv</sub>: Extract solution volume, 1 mL;

N: Amount of cells, 10<sup>4</sup>.

### B. 96 well flat-bottom plate

#### 1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every mg of protein.

$$\begin{aligned}\text{TrxR (U/mg prot)} &= [\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (V_s \times C_{pr}) \div T \\ &= 245 \times [\Delta A(T) - \Delta A(B)] \div C_{pr}\end{aligned}$$

#### 2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every gram of sample.

$$\begin{aligned}\text{TrxR (U/g)} &= [\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (V_s \div V_{sv} \times W) \div T \\ &= 245 \times [\Delta A(T) - \Delta A(B)] \div W\end{aligned}$$

#### 3. Cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of TNB at 37°C (mammal), 25°C (other) per minute every 10<sup>4</sup> cell.

$$\begin{aligned}\text{TrxR (U/10}^4\text{cell)} &= [\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (N \div V_{sv} \times V_s) \div T \\ &= 245 \times [\Delta A(T) - \Delta A(B)] \div N\end{aligned}$$

ε: TNB molar extinction coefficient at 412 nm, 1.36 × 10<sup>4</sup> L/mol/cm;

d: Light path of cuvette, 0.6 cm;

V<sub>rv</sub>: Total reaction volume, 200 μL = 2 × 10<sup>-4</sup> L;

V<sub>s</sub>: Supernatant volume (mL), 0.02 mL;

C<sub>pr</sub>: Sample protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time (min), 5 minutes;

W: Sample weight (g);

V<sub>sv</sub>: Extraction volume, 1 mL;

N: Amount of cells, 10<sup>4</sup>.

#### Note:

1. Dilute 5 times with distilled water when detecting mammalian tissue and blood samples, detect quickly as soon as possible.
2. Because the extract solution contains a certain concentration of protein (about 0.1 mg/mL), the protein content of the extract solution itself needs to be subtracted when determining the protein concentration of the sample.

#### Experimental instances:

1. Take 0.1g of Chinese rose petals, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4°C, take the supernatant and place it on ice for test according to the measured steps. Calculate  $\Delta A_T = A_4 - A_3 = 0.8600 - 0.8177 = 0.0423$ ,  $\Delta A_B = A_2 - A_1 = 0.0792 - 0.0727 = 0.0065$ ,

calculate the enzyme activity according to sample weight:

$$\text{TrxR (U/g weight)} = 147 \times (\Delta A_T - \Delta A_B) \div W = 5.26 \text{ U/g weight.}$$

2. Take 0.1g of liver, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4°C, take the supernatant and place it on ice for test according to the measured steps. Calculate  $\Delta A_T = A_4 - A_3 = 0.8538 - 0.2102 = 0.6436$ ,  $\Delta A_B = A_2 - A_1 = 0.0792 - 0.0727 = 0.0065$ , calculate the enzyme activity according to sample weight:

$$\text{TrxR (U/g weight)} = 147 \times (\Delta A_T - \Delta A_B) \div W \times 4 \text{ (dilution ratio)} = 3746.148 \text{ U/g weight.}$$

### Recent Product citations

[1] Li B, Li D, Jing W, et al. Biogenic selenium and its hepatoprotective activity[J]. Scientific reports, 2017, 7(1): 1-11.

[2] Zhang L, Fan J, He J, et al. Regulation of ROS–NF-κB axis by tuna backbone derived peptide ameliorates inflammation in necrotizing enterocolitis[J]. Journal of cellular physiology, 2019, 234(8): 14330-14338.

### Related products:

BC1170/ BC1175	Reduced Glutathione (GSH) Assay Kit
BC1180/ BC1185	Oxidized Glutathione (GSSG) Assay Kit
BC1190/ BC1195	Glutathione Peroxidase (GPX) Assay Kit
BC0350/ BC0355	Glutathione S-transferase (GST) Activity Assay Kit